REMARKS

Applicant respectfully requests reconsideration of the present application in view of the foregoing amendments and in view of the reasons which follow.

Applicant believes that the present application is now in condition for allowance. Favorable reconsideration of the application as amended is respectfully requested.

The Examiner is invited to contact the undersigned by telephone if it is felt that a telephone interview would advance the prosecution of the present application.

Respectfully submitted,

Date June 4, 2001

By _____

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Should additional fees be necessary in connection with the filing of this paper, or if a petition for extension of time is required for timely acceptance of same, the Commissioner is hereby authorized to charge Deposit Account No. 19-0741 for any such fees; and applicant(s) hereby petition for any needed extension of time.

VERSION WITH MARKINGS TO SHOW CHANGES MADE:

In the first full paragraph on page 31 and ending on page 32:

BRIEF DESCRIPTION OF THE FIGURE

Figure 1A is the amino acid sequence (SEQ ID NO: 1) of the FRS2 protein isolated from NIH 3T3 cells. Four tryptic peptides are underlined in the amino acid sequence. The myristylation sequence is underlined with a hatched line at the N-terminus. The portion of the FRS2 protein corresponding to the phosphotyrosine binding domain (PTB domain) is boxed in the figure. Putative SH2 binding regions of FRS2 are indicated in bold.

In the second full paragraph of page 32:

Figure 1B aligns the sequence of the PTB domain of another adaptor protein, IRS-1, (SEQ ID NO: 2) with that of FRS2 (SEQ ID NO: 3). Secondary structural elements (including α-helices and β-sheets) are boxed. Vertical lines report identical amino acids shared between the two proteins. Only two contiguous amino acids are identical within the two proteins.

In the first full paragraph on page 38 and ending on page 39:

cDNA cloning of FRS2: A pair of degenerate primers was synthesized based upon the amino acid sequence of tryptic peptide #2 (SEQ ID NO: 4) (VYENINGLSIPSASGV). PCR was performed with these two primers using cDNA prepared from mRNA isolated from 3T3 cells. The 60 base pair long product of this reaction was sequenced and found to have the correct amino acid sequence. A second round of PCR was then performed with the same cDNA using one primer chosen from the sequence of the initial 60 bp reaction product, and a second degenerate primer based upon the sequence of tryptic peptide #1 of FRS2 (SEQ ID NO: 5) (FVLGPTPVQK); this reaction gave a 170 base pair product. A third round of PCR

was performed with one primer from this 170 base pair product and a T3 primer from the Bluescript vector. The 1.2 kb product of this reaction contained the sequence of peptide #1. Finally, the 1.2 kb fragment was used as a probe for screening a λ cDNA library generated from Swiss 3T3 cells (Stratagene). Two phage clones of λp90-1 and λp90-2 were isolated and further analyzed. Determination of the deduced amino acid sequences of these two clones revealed a long open reading frame (ORF) that contained the sequences of the four tryptic peptides that were isolated from purified FRS2.

In the first full paragraph on page 44 and ending on page 45:

EXAMPLE 4: cDNA CLONING OF FRS2

Peptide sequences from FRS2 were used to design oligonucleotides for amplification by polymerase chain reaction (PCR) of cDNA prepared from mRNA isolated from NIH-3T3 cells. A 1.2 kb PCR product contained the sequences of proteolytic peptide #1 (FVLGPTPVQK) (SEQ ID NO: 5) and peptide #2 (VYENINGLSIPSASGV) (SEQ ID NO: 4) from FRS2. The 1.2 kb PCR product was then used as a probe for screening a cDNA library from NIH-3T3 cells. Two overlapping clones, λp90-1 and λp90-2, were isolated. Determination of the nucleotide sequences of the two clones demonstrated that λp90-2 contained the sequences of all four tryptic peptides isolated from FRS2 and that λp90-2 represents a full-length cDNA clone of FRS2.

In the first full paragraph on page 45 and ending on page 46:

The deduced amino acid sequence of FRS2 determined from clone λp90-2 is presented in Figure 1A. The coding sequence of FRS2 begins at nucleotide number 308. The first methionine is within a Kozak consensus sequence, and is followed by an open reading frame (ORF) of 1527 base pairs, ending with a stop codon at nucleotide 1834. This ORF encodes a protein containing 508 amino acids with a predicated molecular mass of 56,800 daltons. The sequence of FRS2 contains a consensus myristylation sequence (MGXXXS/T)

at the amino terminus of the molecule MGSCCS (SEQ ID NO: 6). Resh, 1994, Cell 76:411-413. In addition, FRS2 contains a stretch of 120 amino acids (residues 11 to 139) with 29% sequence identity to the PTB (phosphotyrosine binding) domain of IRS1 (Figure 1B). Sun et al., 1991, Nature 352:7377. It has been shown that the PTB domain of IRS1 binds to a tyrosine phosphorylated NPXYp sequence in the juxtamembrane region of the insulin receptor. White et al., 1988, Cell 54:641-649. FRS2 also contains two potential Grb2 binding sites (NYEN (SEQ ID NO: 7) and NYVN (SEQ ID NO:8)) (Figure 1B).